

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark
Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day month year) 21 June 1999 (21.06.99)	
International application No. PCT GB98 03034	Applicant's or agent's file reference IPD P1183 WOD
International filing date (day month year) 09 October 1998 (09.10.98)	Priority date (day month year) 25 October 1997 (25.10.97)
Applicant SQUIRRELL, David, James et al	

1. The designated Office (hereby notified) is the following:

☒ in the demand filed with the International Bureau on 10 May 1999 (10.05.99)

10 May 1999 (10.05.99)

☐ in a notice effecting later election filed with the International Bureau on2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

C. Carrie

Facsimile No. 01 22 74 12 44

Telephone No. 01 22 74 85 45

INTERNATIONAL SEARCH REPORT

National Application No.

PCT/GB 98/03034

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/53 C12N9/02 C12P21/02

According to International Patent Classification (IPC), or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 030 563 A (NASOFF M ET AL) 9 July 1991 see examples I-III ---	10
A	BELINGA, HONORE FAUSTIN ET AL: "Firefly luciferase purification using polyethylene glycol and Dyematrix Orange A" J. CHROMATOGR., A (1995), 695(1), 33-40 CODEN: JCRAEY, XP004022931 ---	
A	WO 94 17202 A (SECR DEFENCE BRIT ;SQUIRRELL DAVID JAMES (GB)) 4 August 1994 ---	
A	WO 96 22376 A (SECR DEFENCE ;SQUIRRELL DAVID JAMES (GB); LOWE CHRISTOPHER ROBIN () 25 July 1996 -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex

Special categories of cited documents

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

13 January 1999

Date of mailing of the international search report

20/01/1999

Name and mailing address of the ISA

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NL - 2280 HV Rijswijk
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Authorized officer

Espen, J

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/03034

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5030563	A	09-07-1991	NONE	
WO 9417202	A	04-08-1994	AT 170222 T	15-09-1998
			AU 677627 B	01-05-1997
			AU 5841794 A	15-08-1994
			BR 9406417 A	26-12-1995
			CA 2154462 A	04-08-1994
			CN 1119029 A	20-03-1996
			DE 69412790 D	01-10-1998
			EP 0680515 A	08-11-1995
			ES 2120006 T	16-10-1998
			GB 2289945 A,B	06-12-1995
			HU 72396 A	29-04-1996
			NO 952917 A	06-09-1995
			NZ 259576 A	24-06-1997
			US 5648232 A	15-07-1997
WO 9622376	A	25-07-1996	AU 4397396 A	07-08-1996
			CA 2210354 A	25-07-1996
			EP 0804587 A	05-11-1997
			GB 2311525 A,B	01-10-1997
			JP 10512750 T	08-12-1998
			NO 973349 A	19-09-1997

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/53, 9/02, C12P 21/02	A1	(11) International Publication Number: WO 99/22004 (43) International Publication Date: 6 May 1999 (06.05.99)
(21) International Application Number: PCT/GB98/03034 (22) International Filing Date: 9 October 1998 (09.10.98) (30) Priority Data: 9722481.0 25 October 1997 (25.10.97) GB (71) Applicant (for all designated States except US): THE SECRETARY OF STATE FOR DEFENCE [GB/GB]; Defence Research Agency, Ively Road, Farnborough, Hampshire GU14 0LX (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): SQUIRRELL, David, James [GB/GB]; DERA Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). PRICE, Rachel, Louise [GB/GB]; DERA Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). MURPHY, Melanie, Jane [GB/GB]; DERA Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). (74) Agent: BOWDERY, A., O.; D/IPR, Formalities Section (DERA), Poplar 2, MOD Abbey Wood #19, Bristol BS34 8JH (GB).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: EXPRESSION SYSTEM (57) Abstract A method for producing a polypeptide product which is substantially free of an undesired protein, the process comprising culturing a host cell which is able to express said polypeptide product and which is able to express said undesired protein only in a mutant form which form has the activity of the corresponding native protein under culture conditions but is unstable under conditions at which the said polypeptide product remains stable; and recovering the desired product, wherein either the host cell culture or the recovered product is subjected for a sufficient period of time to conditions under which the undesired protein is unstable so as to denature the undesired protein. The method allows the production of products which are substantially free of particular undesired contaminants.		

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

16

REC'D 18 JAN 2000

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Applicant's or agent's file reference IPD/P1183/WOD		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
FOR FURTHER ACTION		
International application No. PCT/GB98/03034	International filing date (day/month/year) 09/10/1998	Priority date (day/month/year) 25/10/1997
International Patent Classification (IPC) or national classification and IPC C12N15/53		
Applicant THE SECRETARY OF STATE FOR DEFENCE et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 10/05/1999	Date of completion of this report 11.01.00
Name and mailing address of the international preliminary examining authority  European Patent Office D-80298 Munich Tel +49 89 2399 - 0 Tx 523656 epmu d Fax +49 89 2399 - 4465	Authorized officer Huber, A Telephone No +49 89 2399 8173



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB98/03034

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-8 as originally filed

Claims, No.:

1-18 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-17
	No:	Claims	18
Inventive step (IS)	Yes:	Claims	1-17
	No:	Claims	18
Industrial applicability (IA)	Yes:	Claims	1-18
	No:	Claims	

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB98/03034

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB98/03034

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application is concerned with a method for producing a polypeptide which is substantially free from an undesired protein, wherein the undesired protein is expressed from a host cell only in a mutant form which is unstable under conditions at which the desired polypeptide remains stable.
The exemplified polypeptide is luciferase and the undesired contaminant is adenylate kinase.

2. Reference is made to the following documents:

D1: WO-A-94/17202

D2: BELINGA, HONORE FAUSTIN ET AL: 'Firefly luciferase purification using polyethylene glycol and Dyematrix Orange A' J. CHROMATOGR., A (1995), 695(1), 33-40

3. D1 discloses a method for determining the presence/amount of microorganisms present in a sample comprising estimating the amount of adenylate kinase therein. It also discloses reagents including adenylate kinase free luciferase.

D2 discloses a method for purifying luciferase that permits removal of enzymes (adenylate kinase) interfering in the bioluminescent assay.

4. None of the documents cited in the international search report discloses or renders obvious a method for producing a polypeptide which is free of undesired contaminant proteins, wherein the gene encoding the undesired protein is mutated such that it is only expressed under culture conditions but unstable under conditions at which the desired polypeptide remains stable, e.g. at elevated temperature or pH conditions.

The subject-matter of Claims 1-17 is therefore considered as novel and inventive (Art 33 (2) and (3) PCT).

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB98/03034

5. Claim 18 relates to a polypeptide which is substantially free of an undesired protein, as obtained by a method according to any of Claims 1-9.

The claim is formulated as a product-by-process. The attention of the applicant is drawn to the fact that a product is not rendered novel merely by the fact that it has been produced by means of a new process. Proteins which are free from undesired proteins are amply known from the prior art, see for example D2, which describes a method for producing luciferase which is substantially free of adenylate kinase. A protein which is produced and recovered according to the method of present invention cannot be distinguished from the same protein produced by a different method.

Therefore, the subject-matter of Claim 18 is not regarded as novel (Art. 33(3) PCT).

Re Item VIII

Certain observations

1. The following expressions found in the claims are vague and unclear and as such render the scope of the claims open to interpretation (Art. 6 PCT):
- i) Claims 1 and 18: substantially free of;
 - ii) Claim 4: elevated temperatures (elevated in comparison to which temperature?)
 - iii) Claims 13 and 14: a cell which comprises a cell

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference IPD/P1183/W0D	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA 220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 98/ 03034	International filing date (day month year) 09/10/1998	(Earliest) Priority Date (day month year) 25/10/1997
Applicant THE SECRETARY OF STATE FOR DEFENCE et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. ☐ **Certain claims were found unsearchable** (see Box I).

2. ☐ **Unity of invention is lacking** (see Box II).

3. ☐ The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

☐ filed with the international application.

☐ furnished by the applicant separately from the international application.

☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed

☐ Transcribed by this Authority

4. With regard to the **title**, ☒ the text is approved as submitted by the applicant

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

Figure No. ☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 98/03034

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/53 C12N9/02 C12P21/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched: classification system followed by classification symbols

IPC 6 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	BELINGA, HONORE FAUSTIN ET AL: "Firefly luciferase purification using polyethylene glycol and Dyematrex Orange A" J. CHROMATOGR., A (1995), 695(1), 33-40 CODEN: JCRAEY, XP004022931 ---	
A	WO 94 17202 A (SECR DEFENCE BRIT :SQUIRRELL DAVID JAMES (GB)) 4 August 1994 ---	
A	WO 96 22376 A (SECR DEFENCE :SQUIRRELL DAVID JAMES (GB): LOWE CHRISTOPHER ROBIN () 25 July 1996 -----	

☐ Further documents are listed in the continuation of box C☒ Patent family members are listed in annex

Special categories of cited documents

- A: document defining the general state of the art which is not considered to be of particular relevance
- E: earlier document but published on or after the international filing date
- L: document which may throw doubts on priority (claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- O: document referring to an oral disclosure, use, exhibition or other means
- P: document published prior to the international filing date but after than the priority date claimed

- T: later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- X: document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- Y: document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- Z: document member of the same patent family

Date of the actual completion of the international search

13 January 1999

Date of mailing of the international search report

20/01/1999

Name and mailing address of the ISA

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Fax: +31-701 340-3016

Authorized officer

Espen, J

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/GB 98/03034

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5030563	A	09-07-1991	NONE	
WO 9417202	A	04-08-1994	AT 170222 T	15-09-1998
			AU 677627 B	01-05-1997
			AU 5841794 A	15-08-1994
			BR 9406417 A	26-12-1995
			CA 2154462 A	04-08-1994
			CN 1119029 A	20-03-1996
			DE 69412790 D	01-10-1998
			EP 0680515 A	08-11-1995
			ES 2120006 T	16-10-1998
			GB 2289945 A, B	06-12-1995
			HU 72396 A	29-04-1996
			NO 952917 A	06-09-1995
			NZ 259576 A	24-06-1997
			US 5648232 A	15-07-1997
WO 9622376	A	25-07-1996	AU 4397396 A	07-08-1996
			CA 2210354 A	25-07-1996
			EP 0804587 A	05-11-1997
			GB 2311525 A, B	01-10-1997
			JP 10512750 T	08-12-1998
			NO 973349 A	19-09-1997